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Original Research

Is double frozen blastocyst transfer better than sequential single frozen blastocyst transfers in womenover >35 years of age?

Freeze-all blastocyst transfer cycles women aged >35

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Abstract
Aim: This study aimed to assess live birth rates after double blastocyst transfer versus sequential single blastocyst transfers after freeze-all cycles in women aged over 35 years.
Material and Methods: Between January 2016 and December 2018, we conducted a retrospective analysis of 296 women over 35 years of age who had all their embryos frozen and subsequently transferred. The inclusion criteria were women over 35 years of age who had their entire cohort of embryos frozen at the blastocyst stage for different indications based on physician preference. All patients had at least two good-quality embryos in their frozen cohort. Preimplantation genetic testing cycles were excluded from the study. Demographic data were extracted from patient files and electronic databases. Women were categorized based on their mode of embryo transfer (D-FBT vs. SS-FBT). The couple decided to adopt one or more strategies after consultation. The study's primary outcome was the live birth rate (LBR) per woman after one double versus two sequential single embryo transfers.
Results: Overall, 296 women underwent 362 embryo transfer cycles (D-FBT=186; SS-FBT=176). When adjusted for female age, the cumulative LBR per woman was similar in D-FBT (46.2%) and SS-FBT (58.2%) (p=0.054, aHR=1.62, 95% CI:1.00-2.60). While one monozygotic twin delivery was observed in the SS-FBT group, 22 of 86 (25.6%) live births after D-FBT were twins.
Discussion: Following a freeze-all cycle, SS-FBTs yielded similar live birth rates (LBR) as D-FBT in women aged over 35 years.

Keywords
Freeze-all, Embryo Transfer, Blastocyst, Multiple Pregnancies, In Vitro Fertilization

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Introduction

In the early years of In Vitro Fertilization (IVF), multiple embryos were transferred into the uterus to compensate for low implantation rates. However, advances mainly in the IVF laboratory, including better culture media, more advanced incubators, and particularly embryo vitrification, together with the realization of complications of multiple pregnancies, have reduced the number of embryos recommended for transfer. This paradigm change occurred concurrently with advances in embryo freezing techniques. Embryo vitrification results in excellent post-thaw survival and implantation rates that are equivalent, if not better, than fresh transfers [1]. Freezing all embryos for later transfer quickly became a widespread practice to prevent ovarian hyperstimulation syndrome, hinder the adverse effects of ovarian stimulation on implantation, and correct incidental endometrial pathologies encountered during ovarian stimulation [2, 3].

Currently, it is customary to transfer a single embryo, especially if the quality is good and the patient does not have adverse prognostic factors such as advanced age and previous implantation failures. Vitrification directly contributes to the worldwide acceptance of elective single embryo transfer, which may be the best choice for preventing maternal/fetal complications associated with multiple pregnancies [4-6]. Defining embryo quality for successful elective single embryo transfer (eSET) is fundamental.

According to Turkish IVF legislation, single embryo transfer is mandatory in women under 35 years of age, after which no more than two embryos are allowed. However, a considerable number of patients and physicians prefer embryos to be transferred individually, regardless of age. Although retrospective and prospective studies have compared fresh sequential single embryo transfer versus double embryo transfer, there is a lack of data regarding the outcome of freeze-all cycles.

This study aimed to assess live birth rates after double blastocyst transfer versus sequential single blastocyst transfers after freeze-all cycles in women aged> 35 years.

Material and Methods

Study population and Participants

This study is a retrospective analysis of 362 frozen embryo transfer (FET) cycles in 296 couples undertaken in a single tertiary care private hospital-assisted reproduction center between January 2016 and December 2018. Patients provided consent for anonymous analysis and publication of the gathered data for scientific purposes.

We screened 860 patient files in which the cohort of embryos was cryopreserved at the blastocyst stage for various indications. Of these, 296 women aged 35-45 years who had at least two good-quality blastocysts vitrified on the fifth day were selected. The current Turkish legislation that has been in effect since 2010 allows the transfer of two embryos only after 35 years of age, that is why an older cohort was chosen for analysis [7]. Couples undergoing pre-implantation genetic testing and patients with > 2 prior implantation failures were excluded from the study. Patients were categorized and analyzed based on the mode of embryo transfer [one double frozen blastocyst transfer (D-FBT) versus two sequential single frozen blastocyst transfers (SS-FBT)] (Figure 1).

Demographic and clinical data were extracted from patient files and electronic records.

Interventions

Ovarian stimulation, oocyte retrieval, fertilization, vitrification, and warming

Ovarian stimulation was performed using recombinant follicle-stimulating hormone (FSH) alone or in combination with human menopausal gonadotropin (HMG). Premature luteinizing hormone (LH) surge was suppressed by using a gonadotropin-releasing hormone (GnRH) antagonist in a fixed protocol. The final maturation of oocytes was induced with 250 µg recombinant human chorionic gonadotropin (hCG) or leuprolide acetate in the event of overstimulation. Oocyte retrieval was performed under general anesthesia 35-36 h after the ovulation trigger, using a double-lumen needle. Intracytoplasmic sperm injection (ICSI) was used to fertilize the oocytes.

All included patients had at least two day 5 embryos with grade 3BB and above that were vitrified [8].

Correction of uterine pathology

Patients with endometrial polyps discovered during ovarian stimulation underwent hysteroscopic removal prior to vitrified/warmed embryo transfer.

Endometrial preparation for vitrified/warmed embryo transfer

All vitrified-warmed embryo transfers were performed in an artificial cycle without downregulation. On menstrual cycle day 2 or 3, a baseline transvaginal ultrasound examination was carried out to rule out the presence of a corpus luteum, follicle-s > 10 mm in size, or uterine pathology. If all conditions were met, estradiol valerate tablets (2 mg three times a day) were started. Endometrial thickness, echogenicity, and ovarian activity were checked after 10-12 days. Embryo transfer was planned if the endometrial thickness was at least 8 mm with a triple-line appearance, and there were no follicles measuring 10 mm in size. Embryo transfer was performed on the 6th day after once-daily progesterone gel administration. The dosage was increased to twice daily. All embryo transfers were performed under ultrasound guidance using a Wallace or Cook catheter.

Before embryo transfer, clinicians counseled couples regarding the success and risks of transferring more than one embryo, and encouraged the transfer of a single embryo. The number of embryos to be transferred was determined based on a shared decision between the patient and attending physician.

Statistical evaluation

The collected data were assessed for distribution characteristics using the Kolmogorov-Smirnov test. Continuous variables were defined as median (25^{th}- 75^{th} percentile), and categorical variables were defined as frequency and rate. The two groups were compared using the Mann-Whitney U test for continuous data and the Chi-Square or Fisher's exact tests for categorical data. The primary outcome was the live birth rate per initiated cycle, and the secondary outcome was the multiple birth rate. A generalized estimating equation model and logistic regression analysis were performed to adjust for confounding factors. Some confounders with biological plausibility derived from the literature were selected. Statistical significance was set at a

two-tailed p-value <0.05.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

A total of 362 embryo transfer cycles (176 single and 186 double blastocyst transfers) in 296 women aged over 35 years were analyzed (Figure 2). The mean age of the women was 39 years (range: 36-41). The indications for IVF were male factor in 121, female factor in 97, and unexplained in 78 couples. Freeze-all indications were physician preference (n:219), overstimulation and/or serum progesterone level >1.5 ng/mL on the day of hCG administration (n:61), and endometrial polyps discovered during ovarian stimulation (n:16). Descriptive characteristics were comparable between the D-FBT and sequential SS-FBT groups (Table 1).

Gonadotrophin dose, estradiol level on the day of trigger, number of oocytes collected, number of Metaphase II (MII) oocytes, fertilization rate, and number of vitrified blastocysts on day 5 of embryo culture did not differ between the groups (Table 2).

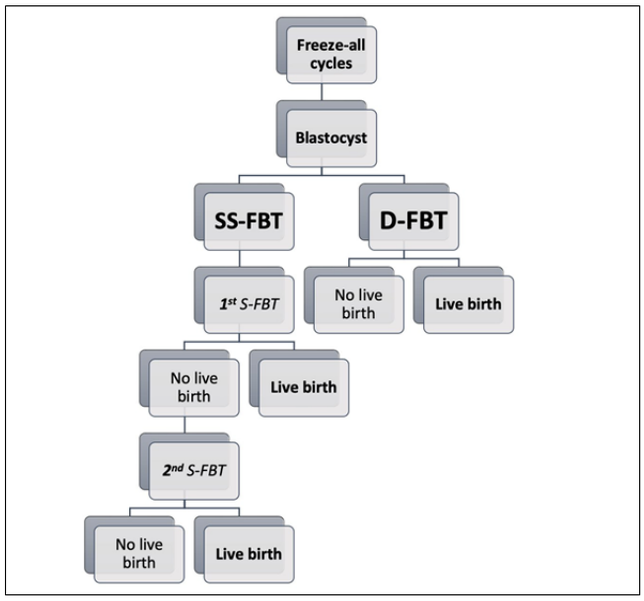

Figure 1. Design of the study

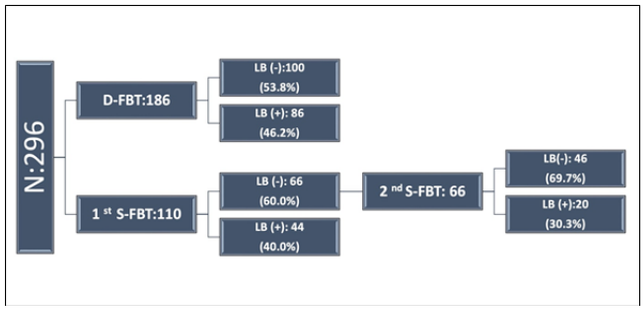

Figure 2. Live birth rates of the patients according to mode of transfer.

Table 1. Demographic characteristics of the patients.

	Double -FBT	Seq-SFBT
Number of women	186	110
Number of embryo transfer cycles	186	176
Female age (years)	39 (36-41)	39 (36-41)
Duration of infertility (years)	3 (2-5)	3 (1-4)
Indications for IVF		
Male	76 (40.8%)	45 (40.9%)
Female	60 (32.2%)	37 (33.6%)
Unexplained	50 (27.0%)	28 (25.5%)
Indications for freeze-all		
Physician preference	137 (73.6%)	82 (74.5%)
Overstimulation or P4>1.5 ng/mL	39 (20.9%)	22 (20.0%)
Endometrial polyps	10 (5.5%)	6 (5.5%)
Number of previous ART cycles	1 (0-2)	1 (0-2)

Values are given as median (25th- 75th percentile) and number (percentage)

Table 2. IVF treatment characteristics of the patients and embryo transfer results.

	Double -FBT	Seq-SFBT	P value
Gonadotropins (IU/day)	300 (225-450)	300 (300-450)	0.398
Peak E_2 (pg/dL)	1576 (1112-2313)	1711 (1132-2444)	0.400
Oocytes retrieved	9 (6-11)	9 (6-12)	0.915
MII (Metaphase II) oocytes	6 (5-9)	7 (4-9)	0.253
Number of 2PN	5 (4-7)	5 (3-7)	0.593
Number of cryopreserved blastocysts	3 (2-5)	2 (2-4)	0.127
Number of embryos transferred (1st/ 2nd cycles)	372/0	110/66	
Pregnancy rate (1st cycle (positive Beta-HCG))	119/186 (64%)	52/110 (47.3%)	0.005*
Preclinical and clinical pregnancy loss (1st cycle)	33/119 (27.7%)	8/52 (15.4%)	0.118
Implantation rate*	141/372 (37.9%)	49/110 (44.5%)	0.210
Live birth after 1st cycle	86/186 (46.2%)	44/110 (40.0%)	0.333
Twin delivery rate (1st cycle)	22/86 (25.6%)	1/44(2.3%) monozygotic twin	0.0005*
Pregnancy rate (2nd cycle)	-	25/66 (37.9%)	
Preclinical and clinical pregnancy loss (2nd cycle	-	5/25 (20.0%)	
Implantation rate	-	22/66 (33.3%)	
Live birth after 2nd cycle	-	20/66 (30.3%)	
Multiple pregnancy rate (2nd cycle)	-	-	
Cumulative live birth rate (1st and 2nd cycles)	86/186 (46.2%)	64/110 (58.2%)	0.054

Values are given as median (25th- 75th percentile) and number (percentage), * denotes statistical significance

Of the 186 women who had transferred two vitrified-warmed blastocysts, 119 (64%) had a positive pregnancy test result, 86 pregnancies resulted in live births (46.2%), and 33 had preclinical and clinical pregnancy losses (Table 2). Of the 110 women who had transferred a single blastocyst, 52 (47.3%) had a positive pregnancy test result, and 44 pregnancies resulted in live births (40.0%). Sixty-six women (58 who failed to conceive and eight who had a pregnancy loss) underwent a second single blastocyst transfer and achieved a pregnancy rate of 37.9% (25/66) and a live birth rate of 30.3% (20/66). Implantation rates (ultrasound verified gestational sac) were 37.9% in the

D-FBT, 44.5% in the first SS-FBT, and 33.3% in the second SS-FBT cycle. While only one monozygotic twin delivery was observed in the SS-FBT group, 22 of 86 deliveries (25.6%) in the D-FBT group were twins. The cumulative live birth rate per initiated cycle was higher in the SS-FBT group (58.2%) than that in the D-FBT group (46.2%). However, when adjusted for female age (the only variable associated with the live birth rate in multivariate analysis), the likelihood of a live birth per cycle initiated was similar between the two groups (p=0.054, aHR=1.62, 95% CI:1.00-2.60).

Discussion

Our results show that when adjusted for female age, SS-FBT results in a similar cumulative live birth rate as one D-FBT in freeze-all cycles. While only one twin delivery was observed with SS-FBT (1 of 44+20; 1.6%), 22 of 86 (25.6%) live births after D-FBT were twins.

IVF success is measured by the rate of a healthy singleton infant delivered with normal weight [5]. eSET is becoming more common because of the recognition of risks associated with multiple pregnancies [9]. In IVF patients with good prognosis, specifically women younger than 37 years of age in their first or second IVF cycle, and when good-quality embryos are available, elective single embryo transfer is recommended by the Practice Committee of the American Society for Reproductive Medicine [10].

eSET results in a higher chance of delivering a term singleton live birth than double embryo transfer (DET). Although this strategy yields a lower pregnancy rate than double embryo transfer in a fresh IVF cycle, this difference is almost entirely overcome by an additional single embryo transfer cycle. The multiple pregnancy rate after eSET is comparable to that observed in spontaneous pregnancies [11]. However, the goal and definition of success can change for couples with prolonged infertility problems. eSET can increase costs, lengthen the time to become pregnant, and reduce the chance of a second child for older couples [12]. Despite all potential adverse maternal outcomes, women older than 35 years are still more inclined to have twin pregnancies [13]. The above notwithstanding, it has been shown that almost 30% of clinicians decide on how many embryos to transfer without consulting their patients. More than one-third of patients do not know if they prefer singleton or twin gestation [14].

One recent study suggested that in cleavage stage freeze-all cycles, eSET should be offered to women aged 35-40 with favorable and average prognosis because the twinning rate can reach more than 20% in these groups; only in the poor prognosis group, DET should be offered [15]. In our study, despite the age of patients > 35 years, the twinning rate was 25.6% in the D-FBT group. However, it should be noted that this group carried favorable characteristics, such as the presence of at least two good-quality blastocysts on day five of embryo development, which cannot be generalized.

Most of the accumulated data regarding the outcomes of single versus double embryo transfers are gathered from the transfer of embryos during fresh IVF cycles. As the freeze-all policy is gaining momentum, it is crucial to know whether transferring sequential single embryos yields equivalent or higher pregnancy rates and lower multiple birth rates than double embryo transfers.

Eum et al. compared the outcomes of double versus sequential single embryo transfer (Seq SET) in both fresh and vitrified-warmed cycles [16]. Single-embryo transfer cycles included both compulsory and elective transfer. They also analyzed the outcomes in terms of female age. In women aged 35 years, pregnancy rates were similar in the elective SET and DET groups (52.0% vs. 54.3%, p = 0.77). Likewise, there was no difference in live birth rates (38.0% vs. 50.0%, p = 0.12). However, the multiple pregnancy rate was significantly higher in the DET group than in the eSET group, regardless of age (p = 0.005 and p = 0.002, respectively). Unfortunately, the study design is prone to introducing several biases that may confound the results. Furthermore, cumulative pregnancy and live birth rates were not assessed.

Park et al. categorized all vitrified-warmed blastocyst transfer cycles into three groups according to the number and quality of blastocysts transferred in women aged > 35 years [17]. This study concluded that to reduce the high number of multiple pregnancies, single good-quality blastocyst transfers are recommended in the freeze-all for patients over 35 years. This study also presumed that SS-FBT would be better than D-FBT in freeze-all cycles over 35 years of age. Another recent study from the same group evaluated patients from all age groups and reached similar conclusions [18].

Long et al. evaluated the effect of blastocyst or cleavage-stage embryo transfers on pregnancy outcomes in 24422 frozen-thawed cycles [19]. According to the results of this study, single-blastocyst embryo transfer appears to be the best choice for all age groups [19].

Monteleone et al. evaluated sequential SET (n:237) versus DET (n:373) strategies in 610 cycles, in which the patients received a fresh or frozen embryo in their first cycles [20]. The success rates were similar between the groups (Sequential SET: 45.9% vs. DET: 46.6%), and the multiple pregnancy rate was significantly lower in the sequential SET (6.7%) group than in the DET (32.2%) group. However, this study differs from ours, as the investigators included cycles in which the patients received either fresh or frozen embryo transfers at the cleavage or blastocyst stages. Furthermore, the patients were relatively younger (Seq SET vs. DET; 33.6-33.9 years, respectively) than those included in our study.

It may be argued that the treatment costs will be lower in the DET group due to the shorter time and fewer cycles utilized to achieve pregnancy. Crawford et al. showed that Seq SET among women younger than 35 years of age resulted in higher treatment costs than DET; however, it also showed that pregnancy/infant-associated medical expenses were markedly lower, resulting in lower overall costs [21]. However, the twinning rate is lower in advanced-aged patients; thus, a cost-effectiveness analysis is needed for this patient group [15].

Conclusion

Our results show that over 35 years of age, SS-FBT has similar LBR success as D-FBT in a freeze-all environment. Thus SS-FBT rather than D-FBT should be offered to women aged > 35 years in the presence of good-quality blastocysts available for transfer. The strengths of this study include the homogenous

patient population and the adjustment for all potential confounders. Limitations are the relatively small number of patients analyzed, the fact that only patients aged > 35 years were included, the unavailability of cost-effectiveness analysis, and the retrospective nature of the study. Future studies should analyze double versus sequential single embryo transfers from the perspective of cost-effectiveness. More conclusive evidence can only be obtained in properly designed large-scale randomized studies.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest
None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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